Interplay between hydrogen and chalcogen bond in cysteine

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Abstract

Protein structures are stabilized by several types of chemical interactions between amino acids, which can compete with each other. This is the case of chalcogen and hydrogen bonds formed by the thiol group of cysteine, which can form three hydrogen bonds with one hydrogen acceptor and two hydrogen donors and a chalcogen bond with a nucleophile along the extension of the C-S bond. A survey of the Protein Data Bank shows that hydrogen bonds are about 40-50 more common than chalcogen bonds, suggesting that they are stronger and, consequently, prevail, though not always. It is also observed that frequently a thiol group that forms a chalcogen bond is also involved, as a hydrogen donor, in a hydrogen bond.

Introduction

It was realized, long ago, that a folded protein "consists of one polypeptide chain which continues without interruptions throughout the molecule (or, in certain cases, of two or more such chains)" and that "this chain is folded into a uniquely defined configuration, in which it is held by hydrogen bonds" [1]. Later on, a series of other non-covalent interactions have been discovered to be responsible for protein folding, stability, plasticity, and function, like van der Waals, hydrophobic and electrostatic interactions [2]. One of these non-covalent interactions has received little attention insofar: it is the chalcogen bond.

It is an attractive interaction between chalcogen atoms (sulfur, selenium or tellurium) and nucleophiles. In molecular moieties like R-X-R (X = S, Se or Te), the nucleophile tends to occupy a position along the extension of one of the R-X covalent bonds (**Figure 1**)[3]. Although recent publications reviewed extensively both theoretical and experimental studies of chalcogen bonds [4][5], minor attention has been paid to this non-covalent interaction in biological systems.

Figure 1. Scheme of the chalcogen and hydrogen bonds that may involve the cysteine side-chain. *Nu* indicates a nucleophi

Despite a pioneering work of Thornton, reporting the interaction between sulfur atoms of cysteine and methionine with the aromatic rings of tryptophan, tyrosine and phenylalanine, which are nucleophiles [6], little was published in the early days of structural bioinformatics. No trace of chalcogen bonds involving methionine sulfur atoms emerged in a 1999 analysis of room-temperature protein crystal structures [7]. In a subsequent study of a larger data set, some evidence of chalcogen bonds between the methionine sulfur atom and backbone or side-chain carboxylate oxygen atoms was observed [8]. Further statistical analyses, coupled with molecular orbital *ab initio* calculations, confirmed that the sulfur atoms of cysteine and methionine can form chalcogen bonds with protein polar atoms [9][10]. More recently, a 2021 study showed numerous chalcogen bonds between the selenium atom of selenomethionine in low temperature protein crystal structures

[11].

Ligand binding to proteins can be influenced by chalcogen bonds, too [12][13]. For example, the activity of ebselen, a glutathione peroxidase mimic, is enhanced by its ability of forming chalcogen bonds with selenium [14]. This non-bonding interaction is important also in the mechanism of inhibition of maltase glucoamylase by salacinol and katalanol [15].

In this manuscript, the chalcogen bonds that involve cysteine side-chains in proteins are identified and compared to the hydrogen bonds that involve the same side-chains, which can behave either as a hydrogen donors or as a hydrogen acceptors (**Figure 1**). This is an attempt to determine the relative energies of the two types of interactions by comparison of their frequency of occurrence.

Results and Discussion

All chalcogen and hydrogen bonds were identified according to the procedures described in the Methods (see below). Interatomic contacts that might be considered, according to the stereochemical criteria used in this study, both hydrogen and chalcogen bonds were discarded (about 20% of the chalcogen bonds may be confused with hydrogen bonds and about 1% of the hydrogen bonds may be confused with chalcogen bonds).

Details about hydrogen and chalcogen bonds are given in Supplementary Material (**Tables S1-S4**) and are only briefly presented here.

As it can be expected, given the high frequency of occurrence – one per residue – the most common nucleophile that forms chalcogen bonds is the main-chain oxygen atom. Surprisingly, sulfur atoms act rather frequently as nucleophiles, too, despite their low frequency of occurrence in proteins [16]. As it was already observed, cysteines tend to be more frequently hydrogen donors that hydrogen acceptors in hydrogen bonds, roughly with a ratio 2:1 [4]. When the cysteine thiol group acts as a hydrogen donor, the hydrogen acceptor is often a main-chain oxygen atom and when it acts as a hydrogen acceptor, the hydrogen donor is frequently a main-chain or a water oxygen.

Table 1. Frequencies of chalcogen bonds (Cb) and Hydrogen bonds (Hb) in the Single data set and in the nine subsets of the Protein Data Bank assembled with the RaSPDB procedure (see Materials and Methods for details). The average values (estimated errors in parentheses) are computed on these nine subsets.

Dataset	Number Cb	Number Hb	% Cb	% Hb
Single	833	31972	2.54	97.46
raspb_1	321	16848	1.87	98.13
raspb_2	347	17141	1.98	98.02
raspb_3	359	17026	2.06	97.94
raspb_4	363	17255	2.06	97.94
raspb_5	346	16878	2.01	97.99
raspb_6	340	17288	1.93	98.07
raspb_7	359	16381	2.14	97.86
raspb_8	348	17171	1.99	98.01
raspb_9	347	16936	2.01	97.99
Average-1-9			2.01(0.03)	97.99(0.03)

Concerning the focus of this study - the relative frequency of chalcogen and hydrogen bonds – it is interesting to observe that hydrogen bonds are about 40-50 times more common than chalcogen bonds (**Table 1**). This suggests that they are stronger. Not 40-50 times stronger, obviously. This indicates that in most of the cases, the hydrogen bond that a thiol group may form is more stable that alternative chalcogen bond: even a small difference would produce the prevalence of hydrogen bond, from a thermodynamic perspective. Precise

estimations of the energy of these interactions is unfortunately impossible, based on statistical observations, since the probability density functions of the energies are unknown. However, it is clear that hydrogen bonds are stronger, on average.

Table 1 also shows that trends and tendencies evaluated by using a non-redundant subset of the Protein Data Bank (*Single*dataset) or by following the RaSPDB method (*raspdb_x* datasets) are nearly equivalent. This reinforces the use of the RaSPDB method, which allows one to use a greater amount of information and to compute estimated errors.

The trends outlined above are independent on the secondary structure or on the degree of solvent accessibility of the cysteines.

If chalcogen and hydrogen bonds would have the same strength, one would expect two-to-three hydrogen bonds per chalcogen bond, since the number of hydrogen bonds that a cysteine thiol group can form is likely to be higher than the number of chalcogen bonds that it can form (**Figure 1**). However, the observed difference in the number of bonds is much higher.

The interaction energy of both chalcogen and hydrogen bonds can be quite variable. Both chalcogen and hydrogen bond have an electrostatic component, which strongly depend on the local environment – *i.e.*on the local dielectric constant. Consequently, chalcogen bonds may be stronger than hydrogen bond, in some cases.

It is interesting to observe that the presence of a hydrogen donor may hinder, because of steric reasons, the formation of a chalcogen bond along the extension of the C-S covalent bond. In other words, the hydrogen donor and the nucleophile roughly compete for the same position close to the sulfur atom (**Figure 1**).

Table 2. Fraction of chalcogen bonds associated with a hydrogen bond involving the SG cysteine atom, in the Single data set and in the nine subsets of the Protein Data Bank assembled with the RaSPDB procedure (see Materials and Methods for details).

Dataset	Fraction (%)
Single	66.3
raspdb_1	67.0
raspdb_2	64.6
raspdb_3	63.5
raspdb_4	66.7
raspdb_5	61.0
raspdb_6	65.0
raspdb_7	69.4
raspdb_8	69.3
raspdb_9	64.8
Average-1-9	65.8(0.9)

Interestingly, the same thiol group of the cysteine side-chain can be involved in both a chalcogen and a hydrogen bond (**Table 2**). About 65% of the chalcogen bonds are associated with hydrogen bonds. An example is shown in **Figure 2** : cysteine 31 (chain A) of human thymidylate kinase forms a chalcogen bond with the main-chain oxygen atom of alanine 37 and forms a hydrogen bond with the main-chain oxygen atom of valine 27 (PDB file 1e9a, [17]).

Figure 2. Example of chalcogen and hydrogen bonds formed by the same cysteine thiol group (data from the file 1e9a of t

It is possible to hypothesize a local structural rearrangement that transforms the chalcogen bond into a hydrogen bond, and *vice versa* . In other words, an oscillation from chalcogen to hydrogen bond. This would optimize the bonding requirements and decrease the entropic cost of protein folding.

It is nevertheless important to observe that the statistical analyses presented in this manuscript cannot provide a quantitative estimation of the energy associated with chalcogen and hydrogen bonds. There are in fact several limitations. For example, although the position of the hydrogen atoms is of crucial importance for defining hydrogen bonds, it is usually unknown in protein crystal structures, especially for acidic and rotatable hydrogen atoms. Moreover, without the hydrogen position, one cannot identify chalcogen bonds that might be formed along the extension of the H-S covalent bond. Moreover, chalcogen and hydrogen bonds involving aromatic rings were not considered in the present study, for the sake of simplicity, though they might participate in protein structure stabilization, by forming both chalcogen and hydrogen bonds.

Further analyses, focused on X-ray and neutron protein crystal structures at extremely high resolution might provide additional information as well as analyses of protein structures determined with alternative methods – e.g. cryo-electron crystallography and nuclear magnetic resonance in solution.

Materials and Methods

Data selection

All data were extracted from the enormous amount of information available in the Protein Data Bank [18][19]. Only X-ray crystal structures determined in the 80-120 K temperature range and refined at a resolution of at least 2.0 Å were retained. This resulted in about 66,500 entries of the Protein Data Bank.

Then two strategies were followed to extract non-redundant sets of data.

On the one hand, the pairwise sequence redundancy was reduced with CD-HIT – maximal percentage of sequence identity of 40% [20] – and the attention was limited to chains containing at least 50 amino acids. This resulted in the *Single* dataset containing about 14,000 protein chains.

On the other hand, the RaSPDB procedure was applied [21]. It consists in creating several subsets of the Protein Data Bank. Each subset must be large enough to be representative of the Protein Data Bank and small enough to avoid internal redundancy. Nine non-overlapping subsets, each containing about 7,000 protein chains made by more than 50 amino acids, were assembled, and all statistical analyses were performed on each of them and then averaged. This procedure allows one to use a much larger fraction of Protein Data Bank and to estimate the standard errors of each estimate. This results in the nine subsets $raspdb\_X$ (X=1-9).

Chalcogen bond detection

In previous studies of chalcogen bonds formed by selenomethionine, the position of the nucleophile relative to the selenium atom was described by means of spherical coordinates [7][11], which require the atomic positions of the C-Se-C triatomic fragment of the selenomethionine side-chain. An analogous approach is impossible here, where the attention is focused on the C-S-H triatomic fragment of cysteine, given that the coordinates of this hydrogen atom are usually unknown, since acidic and rotatable hydrogen atoms are often undetected, even at very high crystallographic resolution or in neutron diffraction studies.

In principle, it is possible to compute the position of these hydrogen atoms by optimizing their interactions with atoms close by [22]. This means by optimizing their hydrogen bonds [23]. Here it is preferable to avoid the computation of the position of these hydrogen atoms, since this would inevitably bias the analysis of chalcogen bonds.

As a consequence, a S-Nu chalcogen bond was simply defined as a contact shorter than 3.4 Å (when Nu is an oxygen atom) or than 3.7 Å (when Nu is a sulfur atom) and colinear or nearly colinear with the C-S bond,

which means that the angle $\alpha = 180°$-(C_β-S_γ-Nu) must be narrower than 25° – note that this threshold is larger than 20°, the value used in chemistry and material science, since it is necessary to consider the lower accuracy of macromolecular crystal structures.

Care was taken to remove from the chalcogen bonds' list the disulfide bonds and the short sulfur-sulfur contacts that may be observed for radiation damaged disulfide bonds [24][25]. Analogously, short sulfur-sulfur contacts resulting from the interactions of the sulfur atoms with the same heteroatom – typically a metal cation – were removed from the chalcogen bonds' list.

Hydrogen bond detection

Potential hydrogen bonds that involve cysteine were identified with HBPLUS [26] and filtered according to the following criteria [27][28]: S-A < 4.3 Å and S-A-AA $> 90°$ when the cysteine is a hydrogen donor; and D-S < 4.1 Å when the cysteine is a hydrogen acceptor. Additional stereochemical criteria that can be used to identify hydrogen bonds and that require the knowledge of the position of the hydrogen atoms were disregarded, since the hydrogen atom position is generally unknown.

Miscellaneous

Solvent accessible surface areas were computed with NACCESS [29] and secondary structure assignments were performed with Stride [30].

Author Contributions

OC designed the procedures, executed all computations, and wrote the manuscript.

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Figure legends

Figure 1 . Scheme of the chalcogen and hydrogen bonds that may involve the cysteine side-chain. *Nu* indicates a nucleophile,*AA-A* a hydrogen acceptor, and *D-H* a hydrogen donor.

Figure 2 . Example of chalcogen and hydrogen bonds formed by the same cysteine thiol group (data from the file 1e9a of the Protein Data Bank).

Supporting Information

Table S1. Nucleophilic atom types that form chalcogen bonds with the cysteine SG atom in the single dataset and in the nine subsets of the Protein Data Bank generated with the RaSPDB procedure. The average values (standard errors in parentheses) are computed on these nine subsets.

Atom	Dataset Single	Dataset raspdb_1	Dataset raspdb_2	Dataset raspdb_3	Dataset raspdb_4	Dataset raspdb_5	Dataset raspdb_6	Dataset raspdb_7	Dataset raspdb_8	Dat rasp
main-chain-O	69.9	65.1	64.3	63.5	63.6	63.6	65.6	67.1	60.9	62.2
Ser-O	1.0	0.0	0.0	1.1	0.6	0.9	1.2	1.7	1.4	1.2
Thr-O	0.6	0.6	0.6	0.3	0.0	1.2	1.5	0.6	1.1	0.6
Tyr-O	1.9	1.6	1.7	2.2	2.2	2.6	1.2	1.7	2.0	3.2
Asn-OD1	3.0	3.1	3.5	1.9	3.6	2.9	3.2	2.5	3.4	4.0
Gln-OE1	1.4	0.9	1.2	1.7	0.6	1.2	1.8	1.4	2.0	1.4
Asp-OD1-OD2	5.3	8.4	8.9	7.8	10.2	8.4	8.2	7.0	10.6	9.5
Gln-OE1-OE2	4.8	4.0	4.6	6.1	3.6	3.8	4.7	4.2	4.0	3.7
Cys-SG	4.2	5.3	4.9	3.1	5.5	4.3	3.8	4.5	3.7	5.2
Met-SD	7.1	9.3	9.2	10.0	8.8	9.2	8.2	7.5	9.5	7.8
HOH-O	0.8	1.6	1.2	2.2	1.4	2.0	0.6	1.9	1.1	1.2

Table S2. Percentages of cases in which the Cys side-chain behaves as a hydrogen-donor and as a hydrogen acceptor in the Single dataset and in the nine subsets of the Protein Data Bank generated with the RaSPDB procedure; the average values are computed on these nine subsets (estimated standard errors in parentheses).

Dataset	donor	acceptor
Single	69.2	30.8
raspdb_1	67.2	32.8
raspdb_2	67.1	32.9
raspdb_3	65.8	34.2
raspdb_4	66.1	33.9
raspdb_5	67.7	32.3

raspdb_6	66.9	33.1
raspdb_7	68.0	32.0
raspdb_8	66.9	33.1
raspdb_9	67.3	32.7
Average-1-9	67.0(0.2)	33.0(0.2)

Table S3. Percentages of observation of various hydrogen-acceptors from cysteine in the Single dataset and in the nine subsets of the Protein Data Bank generated with the RaSPDB procedure. The average values (with estimated standard errors in parentheses) are computed on these nine subsets.

Atom	Dataset Single	Dataset raspb_1	Dataset raspb_2	Dataset raspb_3	Dataset raspb_4	Dataset raspb_5	Dataset raspb_6	Dataset raspb_7	Dataset raspb_8	Dataset raspb_9	D A
main-chain-O	79.7	80.9	80.5	79.2	80.3	81.0	79.9	80.7	79.7	80.3	80
Ser-OG	3.0	2.7	2.9	3.2	3.0	2.7	3.0	2.9	3.1	3.1	2.
Thr-OG1	2.9	2.8	2.8	2.8	2.7	3.1	2.7	2.7	2.9	2.8	2.
Tyr-OH	1.3	1.2	1.2	1.2	1.2	1.0	1.0	1.1	1.1	1.2	1.
Asn-OD1	1.5	1.9	2.0	2.1	2.1	2.1	2.2	2.0	2.3	1.9	2.
Gln-OE1	0.9	0.8	0.9	0.7	0.9	0.8	0.8	0.9	0.9	0.8	0.
Asp-OD1-OD2	2.4	2.1	2.0	2.1	2.2	2.2	2.1	2.0	2.1	2.0	2.
Glu-OE1-OE2	1.9	1.7	1.6	1.7	1.4	1.4	1.4	1.7	1.6	1.6	1.
Cys-SG	6.1	5.7	5.8	6.7	6.0	5.5	6.5	5.9	6.2	6.1	6.
Met-SD	0.3	0.3	0.2	0.3	0.2	0.2	0.3	0.3	0.2	0.2	0.
HOH-O	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.

Table S4. Percentages of observation of various hydrogen-donors to cysteine in the Single dataset and in the nine subsets of the Protein Data Bank generated with the RaSPDB procedure. The average values (with estimated standard errors in parentheses) are computed on these nine subsets.

Atom	Dataset Single	Dataset raspdb_1	Dataset raspdb_2	Dataset raspdb_3	Dataset raspdb_4	Dataset raspdb_5	Dataset raspdb_6	Dataset raspdb_7	Dataset raspdb_8
main-chain-N	39.7	39.6	39.5	40.4	42.2	39.5	38.8	38.1	38.6
Ser-OG	3.0	3.0	3.3	3.6	3.4	3.2	3.4	3.4	3.0
Thr-OG1	2.5	2.4	2.6	2.3	2.2	2.7	2.8	2.4	2.5
Tyr-OH	0.5	0.6	0.8	0.7	0.7	0.5	0.4	0.5	0.6
Asn-ND2	0.6	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0.5
Gln-NE2	0.5	0.7	0.8	0.7	0.4	0.7	0.6	0.6	0.6
His-ND1-NE2	0.6	0.6	0.6	0.4	0.6	0.5	0.6	0.6	0.5
Trp-NE1	0.2	0.5	0.6	0.5	0.5	0.5	0.5	0.5	0.5
Arg-NE-NH1-MH2	1.2	1.2	1.2	1.1	1.1	1.1	1.3	1.3	1.0
Lys-NZ	14.3	12.2	12.8	13.7	12.3	12.5	14.0	13.4	13.6
Cys-SG	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HOH-O	36.9	38.8	37.6	36.2	36.1	38.3	37.3	38.9	38.5